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UTERINE BLEEDING AS AN EARLY SIGN OF PREGNANCY IN THE MONKEY (MACACUS RHESUS), TOGETHER WITH OBSERVATIONS ON THE FERTILE PERIOD OF THE MENSTRUAL CYCLE[1]

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It is the purpose of the present paper to bring together some pertinent information concerning six pregnancies that have occurred in the Carnegie monkey colony during the past twelve months. The more significant point concerns an early diagnosis of pregnancy, which bids fair to hold for all animals having the burrowing type of implantation, including man. The second point, relating to the ovulation time or "fertile period" in the sexual cycle of a menstruating animal, is also of special interest for its bearing on that problem in the human species. The placental bleeding as a sign of pregnancy has already been described (Hartman, 1928, a, b, c); but in the meantime the source of the blood recovered from the vagina during early implantation has been ascertained by histological studies of a crucial case (No. 47, below).

For a better understanding of the mating periods, it has been deemed advisable, even at the risk of tedium, to give the pertinent facts concerning the menstrual histories of the six animals. Animal No. 4, and probably also No. 41, were primiparae, the other four multiparae. The data are summarized in Table I.

Female No. 2. This animal has been discussed in the *American Journal of Obstetrics and Gynecology*, Vol. xv, No. 4, 1928. The essential facts in this case may be summarized briefly as follows: After six sterile matings, at various days of the cycle, a fertile mating was achieved on the ninth to the twelfth day of the cycle. On the seventeenth day after the mating, a slight bleeding began, which continued uninterruptedly for twenty days. This was the first case

[1] Submitted for publication October 15, 1928.

of the "placental sign" encountered in the colony. A young male was born after a gestation of almost exactly six lunar months.

Female No. 4. This case is described in the *Bulletin of the Johns Hopkins Hospital*, Vol. xliii, No. 1, 1928 (see also Hartman, 1928, c). The essential facts are as follows: The female was studied for her menstrual history from the beginning of sexual maturity to her first gestation. One mating, on the sixteenth day of the cycle, was sterile;

TABLE 1

Record of matings of six female rhesus monkeys

ANIMAL NUMBER	INFERTILE MATINGS		FERTILE MATINGS	
	Day of cycle	Male	Day of cycle	Male
2	7	A		
	20	A		
	22	A		
	16	D		
	14	D		
	13	D	9–12	A
4	16	D	10	D
7	11	D		
	16	D, A		
	12	A		
	8	D	11	A
36	14	A	9–10	A
41			10	A
47	13	A		
	23	A	14–16	D

the fertile mating occurred on the tenth day. The placental sign manifested itself from the fifteenth to the thirty-seventh day after mating. From the twenty-second to the twenty-fifth day of gestation the bleeding was more pronounced and simulated a light menstruation. A male baby was born on the one hundred seventy-fourth day of gestation.

Female No. 7. This female had had at least two babies before she was received by us in May, 1925, at the age of seven years. Her

eight months' old baby was suckling at the time and continued to do so for ten months longer. No menstruation was observed until March 10, 1926, when there was a slight flow. She menstruated again on September 5, 1926 and on the same day became severely ill. A second attack, with convulsions and subnormal temperature, occurred October 18, but she again recovered and menstruated October 25. The next periods appeared on November 25, December 26, January 27, 1927, February 23, March 20, April 18, and May 16, the cycles being, therefore, 31, 31, 32, 27, 25, 29, and 28 days, respectively. During the summer the animal was amenorrheic and showed only slight evidence of ovarian activity. The next menses noted were those of October 7 to 10; since she was not examined on October 6 but flowed freely on October 7, it is probable that the flow began on the former date. The fertile mating took place on October 17, on the eleventh day of the cycle. In this case, as in the two previous ones, the fertile mating preceded by several days the low leucocyte count of the vaginal lavage. Thus, 16 unstained and 600 stained leucocytes (cf. Hartman, 1928, a) constituted the count on the day of mating, 20 and 20 three days later, practically zero on the fifth day.

Four matings were sterile: (1) night of February 7–8, 1927, with Male D, eleventh day of cycle, four days before the low point in the leucocyte count. There is no clear reason why this mating should not have been fertile, since the conditions were practically identical with those surrounding the fertile coitus later. It is true that on microscopical examination on the morning of February 8 only a few degenerated spermatozoa and sperm heads were found in the scant cellular debris of the vaginal lumen; but the condition of the spermatozoa recovered from the vagina some hours after the coitus does not seem to have any relation to the success or failure of the act. (2) March 11, sixteenth day of cycle; with Male D two hours, then with Male A two hours. Only a few normal-looking spermatozoa, none motile, were recovered from the vagina. (3) April 1, 1:30 to 3:30 p.m., twelfth day of cycle, male A. Sex skin of female intensely red; non-motile spermatozoa, normal in appearance, recovered from vagina. (4) April 26, eighth day of cycle, Male D. Numerous spermatozoa, some motile, recovered from vagina.

On November 4, eighteen days after the fertile coitus, the twenty-

eighth day after the preceding menstruation, the placental bleeding began and continued for twenty-two days. It was very light except for the temporary more severe flow found at the examination made on November 11.

At forty-two days the uterus was readily palpable as a solid organ "about the size of a hen's egg" and the diagnosis of pregnancy, made by vaginal lavage, was corroborated. Parturition occurred on the one hundred fifty-ninth day *post coitum*.

Female No. 36. This large mature female lost her baby during the voyage from India early in 1927. After she was received by us (April 29, 1927) she menstruated eight times before she became pregnant. The flow varied greatly in intensity and duration; in general it was light and of average length. The observed duration of flow was 6, 6, 4, 4, 6, 3, 3, and 3 days, respectively; but since the animals were examined only every other day, the flow may well have been a day or two longer in each case.

The first observed menstrual cycle (May 2 to June 17, 1927) was forty-six days, the average of the other seven cycles, twenty-five days. There was nothing in the vaginal examination to indicate that the first cycle consisted of two, on the assumption that one menstrual flow may possibly have passed unnoticed. The individual cycles ran as follows: 46, 25, 31, 23, 22, 23, 26 and 24 days, respectively; average of all, 27.5 days.

One mating (Male A) occurred during the night of October 10–11, 1927, on the fourteenth day of the cycle. Ten per cent of the numerous spermatozoa recovered the following morning from the vagina were active. Three days later not a single spermatozoön was recoverable by vaginal lavage. A second mating with the same male, 5 p.m. December 16 to 9 a.m. December 17, the ninth to the tenth day of the cycle, proved effective. Many spermatozoa were recovered, of which only an insignificant number showed even a slight activity. Conception may be dated from December 17.

Thirteen days later, or twenty-two days after the last menstruation, the placental sign appeared (December 30), continued for eight days, and re-appeared on the twenty-first day. On the fifth and the seventh days the flow was considerable, as in a normal though slight menstruation.

On the seventy-fourth day of gestation the notes first record the diagnosis of pregnancy by palpation. On March 12, the eighty-sixth day, the embryo was in the head presentation position, which it maintained throughout gestation. Parturition occurred on the one hundred forty-ninth day of gestation.

Female No. 41. This female was evidently a primipara, received from the importer April 29, 1927. She measured 451 mm. and weighed 3810 grams. Succeeding weights were as follows: May 18, 3640 grams; July 28, 4180 grams; September 28, 4550 grams.

A slight bleeding was noted on May 27, followed by seventy days of amenorrhea. In June, sex swellings appeared in the ventral portion of the sex skin, but the vaginal lavage remained clear, as in amenorrhea. Toward the end of this long interval, however, the sex swellings of the pubic region, as well as in other parts of the sex skin, increased greatly and the vaginal wall showed intense desquamation; both phenomena constitute signs of sexual activity, that is, of follicular growth in the ovary. August 5 to 10 a menstrual flow occurred. This was followed by an eighty-two days' period devoid of menstruation but not of sexual activity; for during this whole period the vaginal wall desquamated abundantly, the leucocyte count remained very low, and the sex skin, hips, and base of tail became greatly swollen and reddened. Several laparotomies of animals under similar conditions have taught us that these phenomena are due to large persistent graafian follicles that probably become atretic. September 12 the lavage was a little discolored but no red blood cells were detectable under the microscope. October 25 to 29 a menstruation took place, preceded by a blanching of the skin, reduction in the swelling, and an increase in the cell content of the vaginal lumen. The next two menstrual cycles appeared to be normal in every way. They were 30 and 26 days in length, respectively, the duration of the flow 3 to 5 days.

Ten days after the menses of December 21 to 23 the female was mated with Male D. No spermatozoa were to be seen in the vaginal lavage. Nineteen days later (January 20), or thirty days after the last menstrual period, red blood cells appeared in the vagina. Since these were not found January 23, the female was mated again with Male A; but this turned out to be superfluous, for on January 25 the bleeding was resumed and continued to February 10, a total span of 21

days. This bleeding proved to be the placental sign. It was very slight throughout, in fact, so slight that, had this been a human case, the flow would have passed entirely unnoticed.

The first entry in the protocol to the effect that the uterus could be palpated as a pregnant organ was on the sixty-fourth day of gestation; a month later the head of the fetus could be made out as "the size of a walnut." As the mother lost weight during gestation, it was not surprising that she should abort, which she did on May 4, on the one hundred twenty-fifth day of gestation, passing a fetus several days dead *in utero*.

Female No. 47. This nursing mother had lost her baby en route from Asia. She was received by us May 13, 1927. She measured 456 mm. in crown-rump length and weighed 4600 grams. By August she had gained 300 grams in weight.

She menstruated ten or possibly eleven times before she conceived. The first interval was one of 57 days; the average of the remainder, 29 days. The average of all the cycles is 32 days. If the first interval of 57 days (May 16 to August 7) were divided into two on the basis that a possible June menstruation had escaped notice (which is not likely), the average would be 28.9 days. The first observed menstruation days were as follows: May 16, August 7, September 14, October 16, November 14, December 14, January 18, February 13, and March 4. The respective lengths of the cycles, therefore, were 57, 26, 35, 32, 29, 30, 33, 26, and 21 days. The fertile mating followed the last and shortest cycle.

The animal was mated three times: (1) October 20, Male A, thirteenth day of the cycle. Numerous spermatozoa were recovered, a few motile. (2) December 29, same male, twenty-third day of cycle. Mating was allowed at this time because she had been "riding" another female just before. (3) March 19 to 21 she was left with Male D, the fourteenth to the sixteenth day of the cycle. On the morning of March 21 only dead spermatozoa were recovered from the vagina; the mating was nevertheless fertile.

In determining the probable date of conception, the following may be said. Unfortunately, the female in question proved rather unreliable as to the leucocyte count, which never dropped to zero and seldom near zero. But in general, the low point came after the sixteenth day

of the cycle, that is, later than the average for other monkeys. The curve of desquamation of the vaginal wall, in its turn, has its high point late in the interval. Hence it is probable that the female in question had a longer sterile period early in the cycle than the average. In consonance therewith, the comparatively late mating (fourteenth to sixteenth day) proved fertile. It is quite reasonable to assume that conception took place on the fifteenth day (March 20).

On April 9, the twentieth day of the cycle, the bleeding from the uterus was first noticed (not examined April 7 or 8). After its continuance had been noted for nine days, that is, on the twenty-ninth day of gestation, the animal was sacrificed, since it seemed desirable to secure an intact uterus to determine the source of the "placental bleeding." The entire animal was injected at physiological pressure with Bouin's fluid, after which the genital tract was dissected out and removed with meticulous care in order to avoid all pressure upon the organ.

SOURCE OF "PLACENTAL BLEEDING"

The uterus measured 31.25 mm. in width between the insertion of the round ligaments, 37 mm. from fundus to cervix, 27.5 in dorso-ventral thickness. The organ was opened with great care. First, the musculature was peeled off from a dorsal section; this revealed surprisingly large masses of blood that later proved to be not blood sinuses but dilated uterine glands gorged with blood. A "window" cut through the placenta revealed a 7 mm. embryo. There were two placental sites, primary and secondary, separated by a narrow strip of chorion running like a ribbon horizontally around the uterus. This narrow zone between the margins of the placentae marked the slit-like uterine cavity, bounded on one side by the chorion and the free edges of the placental discs and on the opposite side by the endometrium itself. Blood was found in this uterine cavity.

From a study of the sections the source of the blood could be easily made out. It reached the lumen of the uterus from the uterine glands, many of which were greatly dilated and full of blood. Much of the blood had undergone hemolysis and doubtless in part was resorbed, in part expelled through the cervix into the vagina. How much of this blood reaches the embryo as nourishment is not clear in the

present case. A detailed description of the structures involved and a discussion of their physiology will be given in another place (Wislocki and Hartman).

DISCUSSION AND SUMMARY

In man and the menstruating monkeys it has been much more difficult to establish the time of ovulation within the sexual cycle than in the lower animals. In the latter there are the definite behavioristic manifestations of "heat," at which time (or soon thereafter) the ova are discharged from the ovary. In the primates there is no such well-defined period of "heat." The idea that "heat" and menstruation are homologous, which prevailed throughout a large part of the past century under the authority of Bischoff, has gradually given way before systematic studies. Leopold studied the ovaries in cadavers of women whose menstrual history was known; Hitschmann and Adler, E. Novak and others correlated the ovarian with the uterine changes on material procured at operation; L. Fränkel, R. Schroeder, and numerous followers kept records of observations of ovaries in relation to menstruation in cases of patients laparotomized for a variety of causes not affecting the menstrual cycle. From this extensive mass of data it appears certain that in women ovulation takes place somewhere near the middle of the cycle; that is to say, from the twelfth to the eighteenth day, the "averages" of authors differing within these limits.

The monkey has offered an opportunity to add other methods to the study of the problems involved: laparotomy may be done at will and repeatedly, as Corner has shown; the vaginal contents may be studied from day to day; the animal may be mated at definite times; she may be sacrificed at a critical stage and perfect histological preparations procured of the reproductive or other organs.

The experience with the six fertile matings reported in this paper (Table I) corroborates the findings of Corner (1923), who recovered an ovum from the tube on the fourteenth and one from the uterus on the seventeenth day of the cycle, and those of Allen (1927), who recovered tubal ova on the tenth and the fourteenth day, respectively. Reasons are given above for assuming that conception days range from the tenth to the fifteenth day of the cycle. It is, of course,

within the realm of possibility that ovulation did not follow as promptly upon copulation as has been set forth above and that the spermatozoa may, in one case or another, have waited for a number of days for the descent of the ovum. My interpretation of the vaginal lavages, however, speaks against this assumption and in favor of an average ovulation date around the tenth or eleventh day of the cycle.

The time of onset of the "placental sign," however, varying as it does from the thirteenth to the twentieth day after the assumed date of conception (Table II), would seem to point to a greater variability in the actual conception date. Table II is offered though it is not quite correct since examination of the animals was made three times a week; but even after correction a considerable variation in the onset

TABLE 2

Day of onset and duration of placental sign

ANIMAL NUMBER	DAY OF ONSET	DURATION
2	17	20 days
4	15	22 days
7	18	22 days
36	13	21 days
41	19	21 days
47	20	Killed on tenth day

of the placental sign would remain to be accounted for. The duration of the placental sign is, however, rather uniform, according to Table II—namely, 21 days.

As to the physiological significance of the hemorrhagic processes which incidentally result in leakage of blood into the uterine lumen and thence into the vagina, little can be said at present. It is probable that the blood forms readily available iron and other kinds of pabulum for the embryo, as has been suspected for the rat, the dog, and man. Indeed, in our preparations the compacta of the endometrium and the chorio-basalis layer of the placenta are in places broken through, allowing maternal blood to enter the intervillous spaces.

Whether the placental sign will be applicable to man will have to be determined by actual observation. It is quite possible that the decidual capsule in which the human ovum becomes buried at a very early stage (*cf.* Streeter, 1927) would prevent the escape of blood into the

uterine lumen. But this objection would theoretically be raised also in the case of the rat, where, nevertheless, the placental sign (Long and Evans, 1920) holds in 100 per cent of all pregnancies. A light bleeding after conception, considered until now as an abortive menstruation, is not at all uncommon in women, as every gynecologist knows. This may be the placental sign sufficiently intense to come to notice. Whether in other cases, where it is not manifest, it yet exists in microscopic amounts, as in our macaques Nos. 2, 41, and 47, should be investigated by the gynecologist. The advantages of a certain and early sign of pregnancy in man are obvious. Theoretically, I should expect it to begin about the time a woman might be expected to menstruate anyway and to continue with diminishing intensity for three weeks.

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ON THE PLACENTATION OF A MACAQUE (MACACUS RHESUS) WITH OBSERVATIONS ON THE ORIGIN OF THE BLOOD CONSTITUTING THE PLACENTAL SIGN[1]

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INTRODUCTION

The following case of pregnancy in a macaque with a description of the uterus and of the placenta is thought to be of interest, because of the fact that the reproductive cycle of the animal had been carefully followed before and after mating. The exact date of coition was known and a diagnosis of pregnancy was made from the study of the vaginal secretion. Pregnancy manifested itself 19 days after mating by a prolonged leakage of blood from the uterus. This, the "placental sign," has been observed in six different macaques (Hartman ('28)). The animal was killed in the present instance and the correctness of Hartman's observation proven. The reproductive tract was obtained under conditions of perfect fixation at the period of maximum bleeding. The uterus and placenta are apparently normal and provide an excellent opportunity for the study of an early stage in the placentation of one of the catarrhine monkeys.

HISTORY AND CLINICAL OBSERVATIONS

Animal No. 47 was received from the dealer May 13, 1927. She had only a short while before made the journey from Asia as a nursing mother; but the baby died soon after arrival. For nearly a year the vaginal lavage of the female was examined, usually three times a week, especially with reference to the amount of desquamation, number and kind of leucocytes, character of cornified or epithe-

[1] Received for publication October 17, 1928.

lial cells, and presence of mucus, after the method detailed in a previous communication (Hartman ('28)). But the animal proved much less reliable in the cyclic variations of the leucocytes than females previously studied, for the leucocyte count never reached quite to zero at any time in the cycle. In general the low point in the leucocyte count came as late as, or later than, the sixteenth day,—that is, at least four or five days later in the cycle than was found to be the case in other females. The animal, therefore, probably had a longer "safe" or sterile period after menstruation than certain other females. In consonance therewith, conception occurred later in this case—presumably on about the fifteenth day of the cycle. The cyclic variations in the amount of desquamation from the vaginal wall, as measured in terms of percentage of sediment in the lavage after settling, more nearly conformed to the norm of the other animals, that is, was highest in the late interval, dropping to the lowest mark around the menstrual period.

The animal menstruated ten or possibly eleven times while in our possession—eleven times, if a possible menstruation took place in June. The latter menstrual period may have been short enough to have escaped our notice, but that is not likely. Moreover, there were no changes in the vaginal content to warrant the assumption of a menstrual period in June. The other menstrual dates (first day of the flow) were: August 7, September 14, October 16, November 14, December 14, January 18, February 13, March 4; hence the length of the periods were: 57, 26, 35, 32, 29, 30, 33, 26 and 21 days, respectively. The fertile coitus occurred after the last and shortest menstrual period.

Female No. 47 was mated three times: (1) October 30 with Male A, the 13th day of the cycle. Examination of a vaginal lavage the next day showed numerous spermatozoa of which only a few were motile. (2) December 29 with Male A, the 23rd day of the cycle. Mating was allowed at this time because she had been "riding" another female just before. (3) March 19–21 she was left with Male D, the 14th to the 16th day of the cycle. On the morning of March 21 only dead spermatozoa were recovered from the vagina, as is usual when females are left for more than one day with the male. The last proved to be the fertile mating.

On April 9 the vaginal lavage showed the presence of blood macroscopically; hence it is probable that the flow began a day earlier. April 6, however, the lavage was still white and contained no red blood-cells whatever. If we take conception to have occurred on March 20, and the first appearance of blood from the pregnant uterus as of April 8, we have an interval of 19 days. As it seemed desirable to secure a case of early pregnancy in order to determine the origin of the vaginal blood (the "placental sign"), as a possible method of diagnosis of pregnancy, it was determined to kill the animal, instead of resorting to surgical removal of the reproductive tract, in order to secure an intact uterus undamaged by pressure or traction. After preliminary laparotomy had established the certainty of the diagnosis, the animal was killed on April 18, the 10th or the 11th day of the bleeding. The animal was killed with ether and the entire body was embalmed with Bouin's fluid to insure perfect fixation.

DESCRIPTION OF UTERUS AND PLACENTA

The abdominal cavity was subsequently opened, so that the uterus could be seen *in situ;* after this procedure the reproductive tract was carefully removed for further dissection. The uterus measured 31 mm. in width between the insertion of the round ligaments, 37 mm. from fundus to cervix, and 27.5 mm. in dorso-ventral thickness. The uterus was highly vascular, considerably more so than in the case of a premenstrual organ.

Since an early embryo was expected, the uterus was dissected with great care. First, the musculature was peeled off from the ventral side and surprisingly large masses of what were thought to be blood-sinuses laid bare in the submucosa (Fig. 4). A window cut through the placenta revealed an embryo, which was removed and photographed (Fig. 5). The embryo, which, according to our data should have reached the twenty-ninth day of development, measured 7.5 mm. in length and corresponded approximately to a human embryo of the fifth week. The uterus was then cut into caudal and cranial halves with a razor-blade. Figure 2 shows the fundic half; Fig. 3, the cervical half of the uterus after this dissection.

On examining the interior of the uterus, the placenta is found to be composed of two discoidal masses. One disk, the primary placenta,

is slightly larger than the other and occupies the ventral half of the uterus. The smaller secondary placental disk is attached to the dorsal wall. The two placental disks occupy nearly the entire uterine cavity (Fig. 1).

On examining the specimen under the low power of a Greenough binocular microscope the demarcation of the two placental masses is sharply distinguishable. Between the two placentae there is a relatively narrow zone of membranous chorion which is not attached to the uterine wall. Instead, there exists in this annular zone a patent lumen of the uterine cavity. The mesometral and antimesometral walls of the uterus beneath the region of attachment of the placental disks appear to contain a large number of coarse blood-vessels which are distended with blood. The placenta itself under low magnification exhibits a basal zone which is whitish in character and an outer zone which is red and extremely vascular in appearance. The fetal surface of the placenta is covered by a richly anastomosing network of delicate fetal blood-vessels which radiate from the embryonic body-stalk, near the center of the primary placenta, towards all parts of the primary and secondary placentae (Fig. 1).

The specimen gives us very interesting data regarding the macaque placenta and fills a gap in the scattered and fragmentary observations of Selenka ('00, '03) and of Strahl and Happe ('05) upon their series of macaque placentae. Like the majority of macaque placentae, the specimen consists of two placental disks: the primary placenta, to which the embryo is attached, and a secondary placenta which receives its blood-supply from the primary one. Instances in macaques in which only a single placental disk is present have been noted by Chapman ('79), Keith ('00), Selenka ('00) and Wislocki ('28). As a rule the placenta is bi-discoidal with the primary placenta markedly larger than the other, as the observations of Selenka ('00), Strahl and Happe ('05) and others testify. In the present specimen the disks are nearly equal in size,—a condition which has also been observed.

The size of the placental disks in the present specimen is noteworthy. The primary and secondary disks extend almost completely around the uterine cavity, leaving only a narrow, annular, membranous chorion between them. This condition is either a transient phase in the course of the growth of the placenta and uterus, or may be typical

of those instances in which the two placental masses are very nearly equal in size. In the younger stages briefly described or illustrated by Selenka ('00) the placental fields are relatively much farther apart, a wide zone of membranous blastocyst intervening between the two placental caps. Moreover, in the earlier stages the blastocyst fills only a fraction of the uterus, touching its dorso-ventral walls and leaving wide communicating portions of the uterine cavity laterally and at the fundic and cervical poles. In the present specimen, by the growth of the placental caps and of the entire blastocyst the uterine lumen has been almost obliterated. Judging from the older uteri observed by Strahl and Happe ('05), Wislocki ('28) and others, the placentae do not remain in close approximation, as in the present specimen, during the further course of gestation, but, with the continued growth of the uterus, tend to become separated gradually by an increasing extent of membranous chorion. At term scarcely more than one-third of the chorion is composed of placenta.

In our specimen the primary placenta occupies the ventral half of the uterus, the secondary placenta the dorsal half. From the series of macaques of Selenka and of Strahl and Happe, in which in ten instances the data are given, we are acquainted with the fact that the primary attachment of the blastocyst occurs with equal frequency either dorsally or ventrally.

One of the most interesting features of our specimen is the arrangement of the fetal vessels on the surface of the placenta. Little attention has previously been given to the course and pattern of these vessels. They constitute a rich network of vessels, both arterial and venous, arising from the body-stalk near the center of the primary placenta and spreading out from there over the primary and secondary placenta as well (Fig. 1). At this period, then, the secondary placenta is supplied from all sides with a host of vessels which emanate from the primary placenta. At a later stage this is evidently no longer the case, for in specimens examined by Strahl and Happe (fetal crown-rump lengths 9 and 11 cm., respectively), as well as in still more mature specimens observed by Keith ('00), Wislocki ('28) and others, the primary placenta is connected with the secondary placenta by only a few large arteries and veins, as a rule no more than three or four. Hence the vascular network, as it is constituted in the present speci-

men, awaits a profound reduction in the number of anastomoses existing between the primary and secondary placenta. The several trunks which eventually persist are known to occur as a leash of vessels extending from the primary placenta to the secondary placenta at one point only (Keith) or connecting the two disks at several different points (Wislocki). The former condition may be found to be more common, for the vessels probably persist on the side of the secondary placenta nearest the primary one. In this connection, it is interesting to point out that in Simiae possessing double placentae the dorsal and ventral disks are symmetrically placed and directly opposite one another in the young stages of development. In the older stages, on the other hand, after the vessels have become reduced, the secondary placenta no longer lies directly opposite the primary one. One margin, namely, that to which the vessels cross invariably lies closer to the larger disk than the other, so that the two disks are eccentrically placed to one another, the larger disk being frankly dorsal or ventral, as the case may be.

The uterus and placenta of the present specimen have been sectioned and stained in haematoxylin and eosin and in iron haematoxylin. The thickness of uterus and placenta is 6 mm., the musculature, lamina basalis and the placenta fetalis each being 2 mm. in diameter, respectively. The primary and secondary placental disks are approximately equal in thickness.

The placenta fetalis is composed of a series of stout, simply branching villi which extend across an open, freely intercommunicating intervillous space to become anchored by broad tips to the lamina basalis (Figs. 1, 8 and 10). The outer two thirds of the villi have been invaded by mesenchyme, while the basal thirds or anchoring portions are composed solely of trophoblast. The portions of the villi which contain mesenchyme are clothed with trophoblast which possesses two to four layers of nuclei and appears to be almost entirely syncytial. Langhans cells can occasionally be distinguished beneath the syncytial outer covering, but in most places the cytotrophoblast seems to have disappeared. On the contrary, the basal portions of the villi are composed almost solely of solid masses of cytotrophoblast or Langhans cells. These cells form not only the anchorage of the villi, but spread out in sheets intermingling with decidua to constitute

the choriobasalis (Fig. 10). The cytotrophoblast composing the basal third and anchorage of the villi is responsible for the pale, streaked appearance of the basal third of the placenta in the unstained gross specimen, in contrast to the fetal surface of the labyrinth which is dark red, due to the presence of the fetal circulation, as well as to a preponderance of maternal blood in that zone of the placenta (Fig. 1). In the stained section of the placenta, on the other hand, the basal portion of the labyrinth is darkest owing to the fact that the cytotrophoblast takes a deep nuclear stain (Fig. 10).

The present specimen fits into the series of Selenka and Strahl and Happe, between those specimens mentioned, but not described by Selenka, which were said to correspond to the human of the 27th to 30th day and a specimen briefly described by Strahl and Happe as corresponding approximately to the fifth week. Strahl and Happe find Langhans cells present in their specimen only towards the anchoring tips of the villi.

The floor of the intervillous space is closed by a slender basal syncytium which is continuous with the syncytium covering the villi and which possesses, for the most part, only a single row of nuclei. The basal syncytium is interrupted in places by the orifices of maternal blood-vessels which connect with the intervillous space. In one of our sections, moreover, there appears to be little doubt that the intervillous space is in direct communication with a large, shallow uterine gland, one wall of which has been entirely eroded, thereby establishing a complete continuity with the intervillous space (Fig. 7). This manner of communication of glands with the intervillous space has been generally denied, the compacta supposedly effectively sealing off the outlets of the glands. And, with this rare exception, this appears to be the case in the present specimen.

The choriobasalis is composed of the cytotrophoblast of the expanded ends of the villi which reach the lamina basalis and intermingle with various elements of the endometrium. Degenerating stroma cells, a few elements resembling decidual cells, many extravasated erythrocytes, numerous leucocytes and large, deeply staining cells arising in the walls of blood-vessels are amongst the cells encountered.

Beneath the choriobasalis there is a zone of compacta followed by a glandular layer. At this stage the two are not everywhere sharply

delimited from one another. The compacta consists largely of spindle-shaped connective-tissue cells arranged in sheets. Many of these cells appear to be undergoing an hypertrophy into oval or polygonal cells which approach the appearance of so-called decidual cells. Between the compacta and the uterine musculature there is a zone of uterine glands which are abundant and large. Many of these glands are filled with extravasated blood (Figs. 1 and 8). Moreover, it is ascertained at this moment that fully two-thirds of the structures filled with blood, described in the gross dissection as blood-vessels lying in the uterine wall beneath the placental disks, prove on microscopic examination to be uterine glands tremendously distended with blood. Although the points of entry of these extravasates into the glands have not actually been seen in the sections at hand, it seems not unlikely that the glands are filled from adjacent, thin-walled veins which are abundant in the glandular layer, as well as in a zone adjoining, between the glands and the uterine musculature. However, the blood may not escape directly from the veins into the glands, for, in a number of sections, clefts which are filled with extravasated blood can be seen in the stroma in the neighborhood of the veins and the glands. Be this as it may, the structures resembling blood-vessels, seen in Figures 1, 2 and 8 beneath the placental sites, prove on microscopic examination to be composed of uterine glands and blood-vessels, besides a smaller number of blood-containing clefts in the tissue.

The glands directly beneath the center of the placental disks appear to be, for the most part, sealed off from the placenta by tissue of the compacta. However, the majority of the glands beneath the placental disks appear to pursue an oblique course towards the placental border, where their orifices finally reach the surface of the endometrium adjacent to the placental margins. This oblique course taken by the uterine glands beneath the placenta was also observed by Strahl and Happe. Through these orifices the escape of blood into the lumen of the uterus can be traced (Figs. 6 and 9) and it is undoubtedly from this source that the vaginal bleeding, observed in the living macaque during the fourth week after the onset of pregnancy, arises. At this stage, there is no decidua vera as in the anthropoids and man, nor does the membranous chorion seal off the surface of the endometrium as in

anthropoids and during later stages in some of the catarrhines. The endometrium beyond the placental borders possesses an unbroken epithelial surface and hypertrophied uterine glands which open freely into a patent uterine lumen. The condition of the endometrium resembles very closely at this period that described by Strahl ('05) and Wislocki ('28) as constant throughout gestation in the platyrrhines.

The musculature of the uterus follows upon the glandular layer. The muscle occurs in stout bundles, the outermost layers pursuing a circular course, the inner ones a longitudinal course, although there is no sharp definition of outer and inner coats, the two blending into one another in interlacing bundles. The inner portion of the musculature is characterized by a large number of arteries, veins and lymph-channels which are surrounded by interweaving strands of muscle.

The margins of the placental disks are thinner than the remainder of the placenta. Moreover, the villi are relatively shorter and fewer in number and the intervillous space correspondingly larger (Fig. 11). The borders of the placental disks are undercut by the uterine cavity (Figs. 9 and 11). From the free margins of the disks the chorion crosses as a delicate membrane from the primary to the secondary placenta, enclosing between it and the endometrium a portion of the uterine cavity. This space is bounded on one side by the chorion and the free edges of the placentae; on the opposite side by the endometrium (Figs. 1 and 9). The uterine glands empty freely on the surface of the endometrium and at the paraplacental border into the uterine cavity (Fig. 6). Extravasated blood is abundantly present in the lumina of the glands, as well as in the uterine cavity to which the blood gains access from the glands (Fig. 9).

The membranous chorion is composed of syncytium possessing one or two rows of nuclei and resting upon a layer of richly vascularized mesenchyme continuous and identical with the mesenchymal sheet covering the fetal surface of the placenta. The plexus of blood-vessels connecting the primary placenta with the secondary one occupies this layer of mesoderm.

The surface of the undercut edge of the placenta is composed of a slender wedge of tissue which constitutes the floor of the placental labyrinth at the periphery of the placental disk (Fig. 11). The apex

of the wedge appears to consist of pure trophoblastic tissue, whereas the base of the wedge, in the recess below the placental margin, is composed of a mixture of fetal and maternal tissues identical with the choriobasalis of which it is a continuation. At the angle where this wedge of choriobasalis meets the endometrium, a number of especially large glands are seen extending up obliquely from beneath the deeper part of the placental disk to open by large orifices into the uterine cavity.

Strahl and Happe ('05) have chosen to emphasize in their specimens the presence of maternal tissue extending up the free overhanging border of the placenta in the form of a wall or circumvallation and to designate it as a capsularis incompleta, inferring that it represents an abortive or transient appearance of a capsularis related to the endometrial capsule found in anthropoids and man. Such an analogy seems far fetched, since implantation in the lower Simiae is central and not eccentric or interstitial as in other forms. In several of their specimens, Strahl and Happe picture a ring of elevated endometrium at the placental border. In our specimen conditions are as described and show no signs of encapsulation (Figs. 1, 9 and 11). By comparison of Strahl and Happe's material with our own, one is led to the conclusion that the structure represented by them is purely an artifact, the result of shrinkage and distortion during fixation. Keeping Strahl's conception in mind, while studying a series of placentae of platyrrhines, a condition of the placental border has been observed (Wislocki ('28)) more nearly resembling the anatomical arrangement described by Strahl and Happe. In Alouatta, namely, the single placental disk invades the endometrium very markedly by the growth of the trophoblast and finally undermines the endometrial surface laterally, leaving a relatively large amount of endometrium surrounding the placental border and extending for a period of time even onto the fetal surface. In the present specimen of a macaque, however, as well as in other representatives of old world monkeys (Wislocki), no structure comparable to the capsularis incompleta of Strahl and Happe has been observed. Moreover, in a young macaque placenta, Duckworth ('07) was unable to find any such circumvallation.

In young macaque placentae, as well as in other catarrhine monkeys, Selenka describes curious sprouts or nests of cells which develop

from the uterine and glandular epithelium underneath and along the borders of the placenta. He believes that these cell-nests which become syncytial in character unite with the plasmoditrophoblast to form ultimately the syncytial covering of the villi. Similar sprouts of cells have been observed in young stages of the placenta in platyrrhines (Wislocki ('28)), but it has been seen how these cell-nests ultimately undergo resorption along the advancing border of the trophoblast. However, we do not share the opinion of Selenka that such glandular or other derivatives of the endometrium contribute towards the ultimate syncytial covering of the villi. In the present specimen there is no evidence of glandular transformation as observed by Selenka or by us in platyrrhines. This is not surprising since the specimen is already rather advanced in its growth and the cell-nests, described previously, occurred only in specimens of presomite and early somite development. This bit of evidence is important, however, for, if the process contributed towards the growth of the villi, as Selenka suggested, its very transitoriness would cast doubt upon such an hypothesis. It remains unexplained, in the absence of a complete series of developmental stages, why Duckworth's ('07) presomite specimen failed to show the uterine and glandular epithelial sprouts described by Selenka in specimens which were intermediate in age between Duckworth's and ours.

DISCUSSION

An extravasation of blood into the uterine cavity during gestation is known to occur in many mammals and in a number of orders there is evidence that such extravasates subserve an important physiological need of the embryo in the form of an available source of iron. In the groups in which the placenta is diffuse and adeciduate, the epithelial surface of the uterus remains entirely or largely intact and the escape of maternal red corpuscles into the uterine cavity has not been observed. In many of the orders which are deciduate, on the other hand, the escape of blood into the uterus is a constant phenomenon. In the carnivora, such as the dog and cat, as well as other representatives, localized extravasates of blood, so-called border haematomata (the green border of the dog, the brown border of the cat), are known to occur along the margins of the zonary placenta. The blood escap-

ing from the maternal vessels to form the border haematomata, remains localized in the uterine cavity between the endometrium and a specialized portion of the chorion, the cells of which engage actively in the resorption of formed maternal erythrocytes or the decomposition products of the same. Such extravasated blood, however, remains definitely localized by the chorion and does not appear to escape diffusely into the uterine cavity, nor into the vagina. Theoretically, the mere presence of more than one fetus in the uterus, as is the rule in the carnivores, combined with the annular form of the placentae, would prevent the leakage of blood into the vagina from any other source than the margins of the placentae in the right and left horns nearest the vaginal outlet.

In the rodents the escape of blood from the endometrium into the uterine cavity appears to occur equally regularly. In the guinea-pig, rat and mouse, extravasates are known to occur into the uterine cavity along the border of the discoidal placentae. In these forms, too, there is an active and easily demonstrable resorption of the maternal blood by the highly phagocytic epithelial cells of the yolk-sac which, through the process of inversion common in many rodents, is in apposition to the endometrial wall and is bathed by any fluid or secretion present in the uterine cavity. The escape of maternal blood into the vagina is theoretically possible in these forms. In fact, Long and Evans ('20) describe bleeding from the vagina in pregnant rats, during the early stages of gestation, designating it as the "placental sign." Furthermore Goldmann ('12) by actual injection of India ink into the blood-vessels of pregnant rats demonstrated the anatomical pathway for the escape of blood from the vessels of the endometrium into the uterine cavity. He described vessels in the endometrium pursuing a course from the mesometral surface of the uterus towards the antimesometral surface. These vessels give off, towards the surface of the endometrium, fine branches which anastomose with one another in a plexiform manner. Certain of these twigs ultimately pierce the cuboidal layer of epithelial cells clothing the endometrium in the antimesometral portion of the uterus, so that the vessels communicate freely with the uterine cavity. Goldmann believes that the openings of these vessels possess a valve-like action and that the amount of blood escaping from them is regulated by the rate at which the blood

is assimilated by the wall of the yolk-sac. The visceral lamina of the yolk-sac, possessing short villi which dip into the extravasated blood, is particularly active in absorbing the maternal blood-corpuscles. The visceral wall of the yolk-sac comes in contact at a very early period of development with the uterine cavity by a disappearance of Reichert's membrane and the parietal wall of the yolk-sac in those animals in which so-called inversion of the yolk-sac occurs. This process of extravasation of blood from the endometrium and resorption of the same by the yolk-sac persists until term. It is quite likely that the external bleeding observed by Long and Evans can be traced to the source revealed by Goldmann.

Hartman has recently called attention to external leakage of blood from the vagina in macaque monkeys during part of the gestation period. In the present specimen this bleeding has been traced to uterine glands distended with blood which has probably entered them from neighboring maternal blood-vessels. Since the glands communicate freely with the uterine cavity at the borders of the placentae, the blood has an opportunity to escape into the uterus and thence into the vagina. The presence of free blood in the uterine glands has been noted in other catarrhine forms. Strahl, who speaks of its presence there, believes, however, that it is resorbed eventually by the uterine glands and that it is not destined to be absorbed by the trophoblast or any part of the chorion; hence that it does not supply the fetus with a store of easily assimilable iron as one might suppose from our knowledge of the carnivores and rodents.

The fate of the extravasated blood, beyond the fraction which escapes into the vagina, is obscure. There is no obvious picture of resorption of formed maternal elements to be seen anywhere, either by the epithelial cells clothing the membranous chorion, or by the fetal elements constituting the choriobasalis beneath the placenta. On the other hand, there is also no valid histological evidence that the blood-corpuscles are being assimilated by the epithelial cells lining the uterine glands, as Strahl suggests.

In our sections much of the blood in the glands, as well as in the uterine cavity, appears unquestionably to be undergoing hemolysis, so that by diffusion in a soluble form it may well be undergoing assimilation by the chorion and may therefore actually represent a

valuable source of nourishment for the fetus. Such an hypothesis of assimilation of iron in soluble form by the chorion is strengthened by observations on the human placenta by Hofbauer ('05), who, by appropriate staining for iron, demonstrated its constant passage from the intervillous space, where it is liberated from disintegrating maternal erythrocytes, into the chorionic villi.

Whether bleeding into the uterus at a certain period of gestation is constant for all of the Simiae we do not know. It is certain that in all of the Simiae, so far examined, extravasates of blood are found at certain periods in the endometrium beneath and around the placental sites, including not infrequently the presence of quantities of blood in the uterine glands. That this blood can escape into the uterine cavity seems certain from anatomical considerations in platyrrhines and lower catarrhines and appears to be fully established in the present instance of a macaque. In the anthropoids and man, on the other hand, where the uterine glands are sealed off to a great extent at an early period by the formation of a decidua vera, as well as by a fusion of the membranous chorion with the decidua, and, finally, by the consideration that the ovum is surrounded by a capsularis, we can not homologize the conditions directly with the observations made in lower monkeys. The possibility of vaginal bleeding from the pregnant uterus in the anthropoids must await further study of the uterus during the first period of gestation.

SUMMARY

1. The placenta of a macaque monkey (Macacus rhesus) is described at a stage when the embryo measures 7.5 mm. crown-rump length. The primary and secondary placental disks are relatively large and nearly of equal size. They occupy all of the uterine wall except an annular strip of endometrium which bounds the uterine cavity. The vascular supply of the placental disks consists of a complex network of blood-vessels issuing from the body-stalk to supply the primary placenta and subsequently the secondary one.

2. The uterine glands are large in the macaque at this period and many of them are filled with extravasated maternal blood which can be traced to the uterine cavity into which it escapes. From the

uterus the blood flows in the living macaque into the vagina where it can be recovered from the vaginal fluid.

3. The placental borders are undercut by the uterine cavity and there is no sign of the presence of a wall or circumvallation of the placental margin by decidual tissue to form a so-called capsularis incompleta as described by Strahl. Large uterine glands open freely into the uterine cavity in the recess beneath the placental margin.

4. There is no decidua reflexa. The uterine cavity is bounded by an intact endometrium bearing epithelium. The glands of the endometrium are not sealed off, but communicate freely with the uterine space. At this stage, moreover, none of the uterine glands are proliferating to form cell-nests or being transformed into syncytium as observed by Selenka in younger stages. The uterine glands do not contribute to the formation of the syncytium lining the intervillous space. One uterine gland which communicates directly with the intervillous space, has been seen in our specimen.

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PLATE 1

Abbreviations: *end.*, endometrium; *m.c.*, membranous chorion; *p.pl.*, primary placenta (ventral); *s.pl.*, secondary placenta (dorsal); *u.b.v.*, uterine blood-vessels; *ut.c.*, uterine cavity; *ut.g.*, uterine glands.

FIG. 1. Drawing of the uterus cut open, showing the structure of the uterine wall and the relations of the primary and secondary placental disks. The two disks are separated from one another by a narrow zone of membranous chorion which encloses an annular slit of the uterine cavity between it and the endometrium. The borders of the placental disks are markedly undercut by the uterine cavity. There is no sign of a marginal circumvallation or fold of endometrium at the borders of the placental disks.

The placenta foetalis can be seen composed of simple, stout villi projecting into the intervillous space. On the surface of the fetal placenta the rich anastomoses of the umbilical vessels are visible. The vessels can be seen emanating from a point near the center of the primary placental disk on the ventral wall of the uterus. It is at this site that the body-stalk of the embryo (which has been removed) attached to the surface of the primary placenta. The drawing shows well the pattern of the vessels spreading out from the primary placenta across the ring of membranous chorion to supply the secondary placenta.

In the wall of the uterus the outlines of blood-vessels and uterine glands can be seen. Microscopic examination of sections reveals that the majority of the round or oval openings in the picture which are black, owing to the presence of blood in them, are uterine glands into which blood has been extravasated and not blood-vessels, as one might suspect on gross inspection of the cut wall of the uterus. The defect in the ventral wall of the uterus was caused by the dissection attendant on the removal of a cap in opening the uterus. × 5.

We wish to express our appreciation to Mr. James F. Didusch for the excellent illustration.

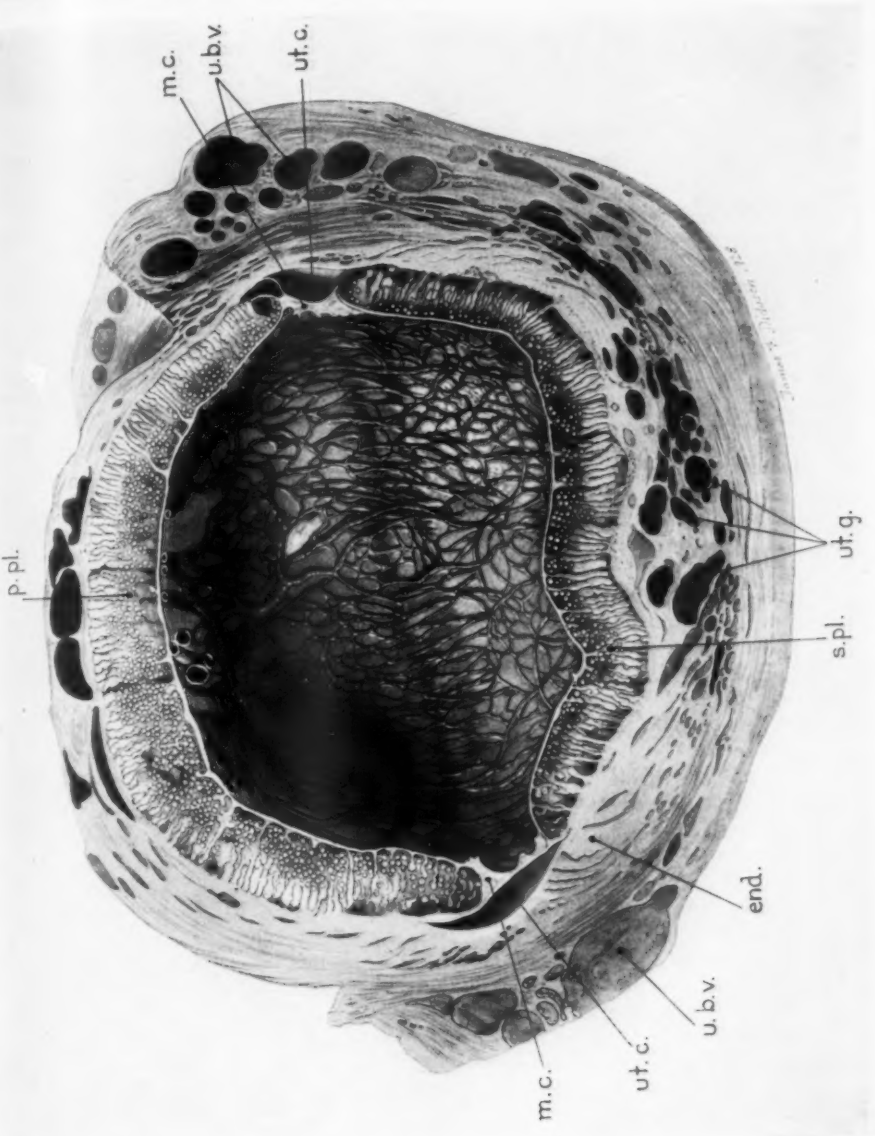
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PLATE 2

FIG. 2. Photograph of the uterus after it had been opened and the embryo had been removed. The view shows the interior of the uterus, looking towards the fundus. To the right the primary placental disk on the ventral wall, to the left the secondary placental disk on the dorsal wall of the uterus. In the right-hand portion of the illustration the margins of the two placental disks can be seen in section with the membranous chorion extending across the uterine cavity from one placental border to the other. The dark slit beneath the chorion between the placental margins is the uterine cavity, containing extravasated blood. In the uterine wall beneath the secondary placental disk, large vessels and uterine glands filled with blood have been cut in section. The defect in the uterus is where a cap of the uterine wall had been removed in opening the uterus. × 2.

FIG. 3. Photograph of the cervical half of the uterus. In the upper part of the picture, against the ventral wall of the uterus, the primary placenta; below, the secondary placenta; and to the left, the slit-like cavity of the uterus and the membranous chorion separating the two disks. On the surface of the primary disk the branches of the umbilical arteries and veins can be seen emanating from a point near the center of the primary disk from where the embryo has been removed. In the wall of the uterus, beneath the secondary placenta, vessels and glands can be seen filled with blood. × 2.

FIG. 4. Photograph of the uterus after removal of a cap of musculature from the ventral wall, showing the hemorrhagic appearance of the sub-placental tissue created by the presence of blood-vessels and of uterine glands distended with extravasated maternal blood. × 2.

FIG. 5. Photograph of the embryo after removal from the uterus. The large yolk-sac can be seen turned back on its stalk, so that it conceals part of the embryo. The embryo measures 7.5 mm. crown-rump length. × 5.

FIG. 6. Photograph of a section, showing the uterine cavity undercutting the margin of the placenta. In the upper part of the picture the placenta foetalis, showing the intervillous space and a few villi. A number of uterine glands can be seen draining into the angle of the uterine cavity. In the uterine cavity at the mouths of the glands is some free blood. In the deeper portion of the section there is a gland markedly distended with extravasated blood. × 47.

FIG. 7. Photograph of a section, showing the choriobasalis below the placental labyrinth, at a point where a large uterine gland communicates freely with the intervillous space. × 47.

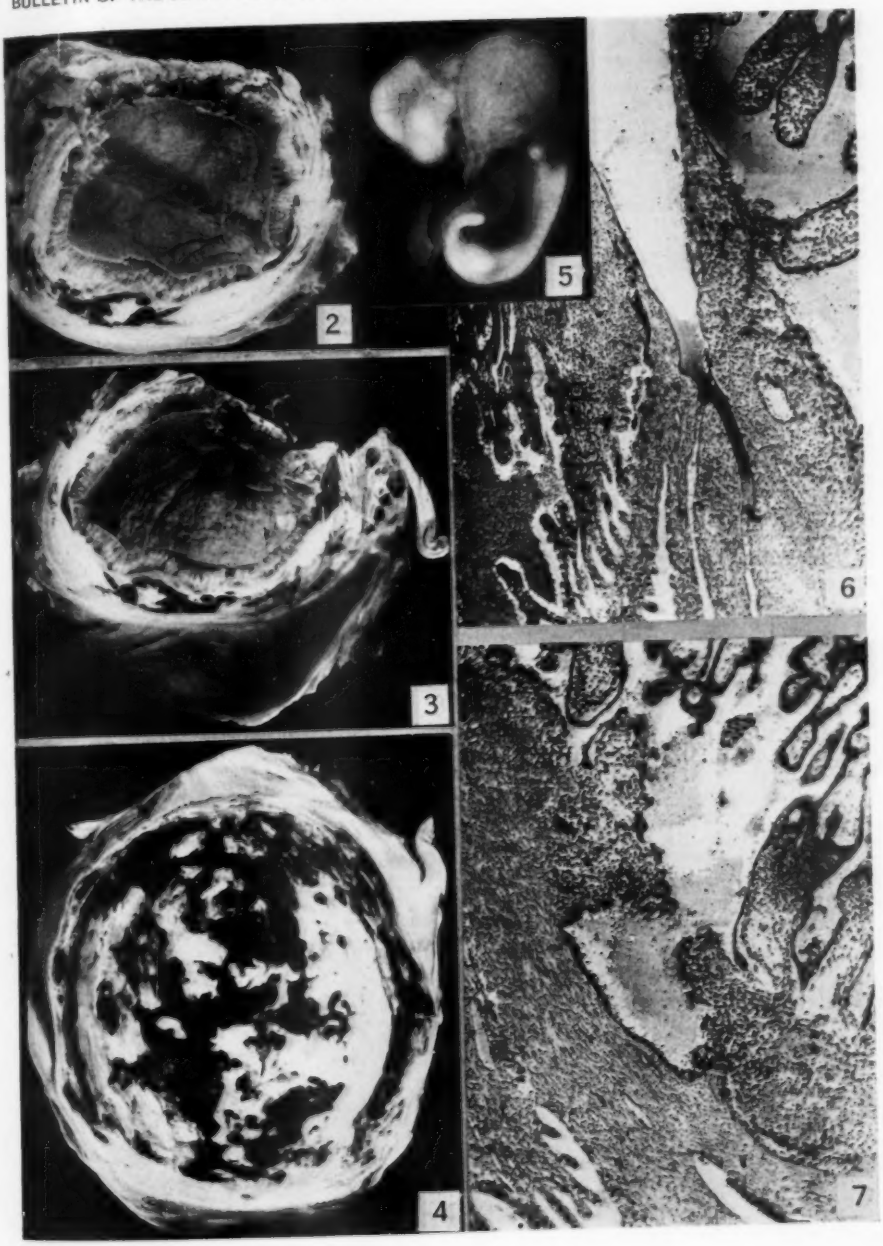
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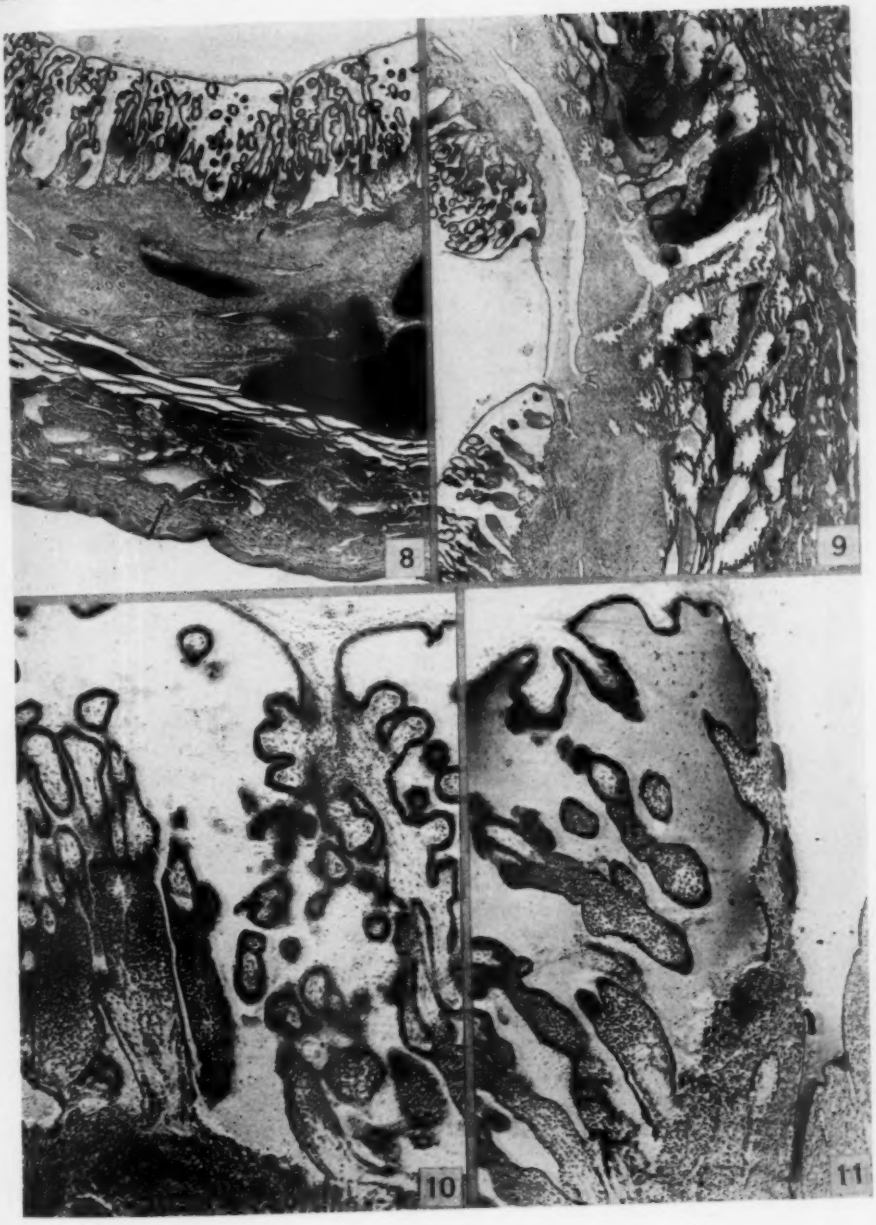
PLATE 3

FIG. 8. Photogragh of a section through the uterine wall and the secondary placenta, showing the character of the placenta foetalis. Between the compacta and the musculature, the uterine glands can be observed, a group of which to the right is distended with blood. × 12.

FIG. 9. Photograph of a section through the uterus between the primary and secondary placental disks. The uterine cavity can be seen as a lense-shaped space bounded below by the endometrium and above by a thin membrane, the chorion, connecting the margins of the two placental disks. Within the uterine cavity extravasated blood can be seen. In the endometrium there are numerous glands, many of which contain free blood. × 12.

FIG. 10. Photograph of a section of the placenta foetalis, showing the villi and the intervillous space. The distal portions of the villi are extremely cellular, consisting of solid masses of Langhans cells, whereas the proximal portions of the villi have been invaded by mesenchyme. × 47.

FIG. 11. Photograph of a section showing the margin of a placental disk. The intervillous space in this region is extensive; the villi relatively short and few in number. The choriobasalis can be seen extending for a short distance up the margin of the placenta which is undercut by the uterine cavity. There is no indication of a circumvallation of the margin of the placenta by a fold of choriobasalis or endometrium. × 47.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THE TETRAPLOID NUMBER OF CHROMOSOMES IN THE MALIGNANT CELL OF THE WALKER RAT SARCOMA NO. 1[1,2]

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The innumerable morphological variations of malignant growths have made them peculiarly unsatisfactory subjects for cytological study. No abnormal cellular structure or modification of cellular structure has been found, common to them all, that could constitute a criterion of malignancy. The most constant characteristics of malignant cells are an increased amount of nuclear material and a more granular cytoplasm. The cytoplasmic granules, which are clearly visible in the living cell, are difficult to preserve and so are seldom seen in the fixed preparation, but the pronounced nucleus is evident in any section of malignant tissue. Whether the increased nuclear content of the malignant cell carries with it a normal or an increased or decreased number of chromosomes has been the subject of prolonged controversy.

As has been shown by W. H. Lewis (1927), the Walker rat sarcoma contains two types of cells; the one small—the mononuclear blood cell—and the other a large spindle cell—the malignant cell. Recently we have observed that the large spindle cell has approximately double the number of chromosomes contained in the small cell. In our observations an effort was made to determine what relation the number of chromosomes in the large spindle cell and in the small cell, respectively, bears to the number in the normal somatic cell of this species of rat, even through at present it cannot be shown to have any definite bearing on the question of malignancy of cells in general.

[1] Part of this investigation was carried on at the Mount Desert Island Biological Laboratory, Salisbury Cove, Maine.

[2] Received for publication October 13, 1928.

MATERIAL AND METHODS

We are indebted to Dr. George Walker for a supply of this tumor, which he first found in 1925 as a spontaneous growth on the under surface of the liver. It had all the characteristics of a malignant spindle-cell sarcoma. Dr. Walker has kept the strain going by implanting every two or three weeks minute pieces of the tumor subcutaneously into young rats. The tumors are capsulated and seldom give rise to metastases. When the implantation is free from contamination, the resulting tumor is composed largely of firm, white, slightly transparent tissue with small opaque (necrotic) areas in the central portion. When the implant is not entirely free from contamination the necrotic areas enlarge, gradually occupying most of the tumor.

The Walker rat sarcoma was first described in 1926 by Lewis and Brüda as a modified white blood-cell tumor and was thought to be composed entirely of this kind of cell, since at that time no other type of cell had been observed in tissue cultures of the tumor. Later, W. H. Lewis (1927a) found that when pieces of the tumor were explanted into chicken plasma an abundant growth of large spindle cells, as well as a proliferation of mononuclear blood cells, took place. The large spindle cell resembled that present in sections of the sarcoma, and Lewis concluded that, although this cell rarely grew in tissue cultures prepared in rat plasma, nevertheless it, rather than the white blood cell, was the malignant cell.

In our tissue culture investigations care was used to select only young firm tumors that had been implanted one or two weeks previously under aseptic conditions. Since we desired to obtain an abundant growth of the malignant cell, most of our cultures were prepared in chicken plasma and other heteroplasms. Only a few were made in autoplasma or in homoplasma, to serve as controls. No nutritive material was added to the medium. A small piece of tumor was cut into many fragments in sterile Locke's solution, after which a few of the pieces were transferred to a dish of 10 cc. of sterile Locke's solution where they were cut into more minute pieces, and from this a single piece, sometimes two were placed on a sterile coverslip. The Locke's solution was withdrawn, a drop of plasma placed on the piece, and the coverslip inverted onto a vaseline ring around the cavity of a hollow-ground slide. The coverglass was sealed to the slide by pressing it

down on the vaseline. The preparations were then incubated at 37.5°C. The cultures were studied on a warm stage of the microscope and when some interesting phases of growth or of karyokinesis were observed, the culture was fixed and stained so that a more detailed picture could be obtained.

Tissue cultures of the thymus, spleen, lymph gland and connective tissue from the same strain of rats were prepared and later fixed in a similar manner, so that karyokinesis of normal somatic cells and of sarcoma cells might be studied under the same conditions.

OBSERVATIONS

I. Cultural characteristics

The characteristics of the growth of mononuclears in cultures of the tumor prepared in rat plasma (Lewis and Brüda, 1926), as well as those of both the mononuclear and the malignant cell in chicken plasma (Lewis, 1927a), have been so carefully described that it is not necessary to give them in detail in this paper. Suffice it to say that the growth of the tumor in chicken or rabbit plasma most nearly approximated that of the tumor *in vivo*, as evidenced by sections made of the neoplasm.

Lewis and Gey (1923) and W. H. Lewis (1927a) have shown that the mass of the sarcoma growth in cultures is made up of two types of cells: one, a mononuclear blood cell and its various transformations, and the other, a much larger cell, the malignant cell. These cells are not peculiar to cultures of the neoplasm, as has been pointed out by W. H. Lewis; sections of the Walker sarcoma No. 1 show it to be composed of large spindle cells, mononuclears, a rich capillary blood supply, and areas of more or less necrotic tissue made up of leucocytes and dead cells. The spindle cell has a highly granular nucleus with a heavily stained, irregular nuclear membrane and nuclear granules, while the mononuclear has an oval or bent, more or less homogeneous nucleus, with a delicately stained nuclear membrane and distinct nucleoli. The endothelial cells forming the walls of the capillaries are not different from such cells in normal tissues. They have a pale cytoplasm with an elongated, mostly homogeneous nucleus containing one or two nucleoli. Both in cultures and in sections of the sarcoma the mononuclear cells appear quite like those found in normal tissue except that

in the cultures in heteroplasma and in the sections they do not exhibit the transformation into macrophages and epithelioid cells so characteristic of them in normal tissue and in cultures of the tumor in autoplasma or in homoplasma. They divide frequently by mitosis, giving rise to two daughter cells of the same type as the mother cell.

Several types of normal tissue—spleen, thymus, lymph node and subcutaneous tissue—gave rise in plasma cultures to a growth containing many mononuclear blood-cells and also some large spindle cells. The one that most nearly resembled that of the sarcoma was the subcutaneous tissue. While in cultures of normal tissue neither type of cell grew as abundantly as in cultures of the sarcoma, nevertheless, it was possible to find numerous mitotic figures both of the spindle cells and of the mononuclear cells, with which to compare those of the cells of the sarcoma cultures.

A comparison of the cultures of normal and of malignant tissue with sections of the sarcoma shows that, although both the spindle cells and the mononuclears of the tumor multiply rapidly and advance side by side as the tumor invades the tissue, cytologically the mononuclear is the same as in normal tissue, while the spindle cell is different, especially in regard to the nucleus (Fig. 1). From this one is led to believe that the sarcoma is not merely a mass of rapidly growing tissue but that the cell which forms the major part of this new tissue is one that has taken on a character different from that of the normal cell. This difference in character becomes even more evident as one compares the mitotic division of the sarcoma cells with that of the normal cells.

II. Mitosis and number of chromosomes

In both cultures and sections of the sarcoma the spindle cell was not only much larger than the other cells and the nucleus proportionally larger, but it also exhibited during division a greater number of chromosomes than the mononuclear cell. In cultures where it was possible to find the cells spread out on the periphery in division, one could observe the individual chromosomes fairly clearly. The smaller ones tended to mass together or to lie below or above larger ones, so that frequently it was difficult to determine the exact number of chromosomes; nevertheless, it was evident that the malignant cell contained twice as many as the mononuclear. Where the dividing malignant cell was side by

side with a non-malignant cell in the same stage of mitosis, as shown in Figures 2 and 5, and 3 and 6, one could readily see this.

The chromosomes in the spindle cells in cultures of normal tissue were not difficult to identify, for these cells were large and the nucleus sometimes almost equaled in size that of the malignant cell of the sarcoma. A number of spread-out spindle cells were found in late prophase and a drawing was made of the chromosomes in each of them. When the chromosomes were counted, they were found to number 40 to 42. This agrees with the observations of Painter (1926) and of Swezy (1926, 1928), who found that the normal somatic cells of the albino rat have 42 chromosomes. In cultures of normal tissue some of the large spindle cells were binucleate, but even so, in none of the cultures of normal rat tissue studies were any cells found with more than the normal number of chromosomes.

The epithelial cell of the thymus had the largest nucleus of any of the normal rat cells grown in plasma cultures, and in a few of them (Fig. 11) the nucleus was slightly larger than that of the malignant cell (Fig. 1). Notwithstanding this, these cells all exhibited approximately 42 chromosomes (Fig. 12).

In cultures of normal tissue the mononuclear blood cells, the macrophages and the clasmatocytes were small and the mitotic figures correspondingly small, but occasionally it was possible to find such a cell in prophase so spread out that the chromosomes could be drawn (Fig. 10). After numerous drawings and counts of the chromosomes of such cells, which ranged in number between 37 and 44, we felt safe in assuming that the number was 42, the same as in other normal somatic cells.

In cultures of the sarcoma, the mononuclear cell was somewhat easier to study, owing to the fact that it frequently divided and many spread-out cells in prophase were thus available for a count of the chromosomes. The number of chromosomes that could be identified varied slightly in the different cells, but all of the cells contained approximately 42.

Dividing malignant cells were abundant in cultures of the tumor in chicken plasma. These were large, as were also the resting cells. The nuclei were frequently twisted or crumpled, so that, as the chromosomes formed, they were located at different levels; it was therefore difficult to be certain that all of them had been identified and that none had been duplicated (Fig. 2). In cultures of the tumor in rabbit

plasma the dividing cells in prophase were particularly well spread out and the chromosomes were therefore more easily drawn (Fig. 13). Numerous drawings and counts were made of the chromosomes of 28 malignant cells at this stage of division. The number identified lay between 76 and 88, usually about 84. Since the individual chromosomes were not smaller than those of the normal cells, it would seem that the increase in number was not the result of fragmentation. It was therefore decided that the malignant sarcoma cell had 84 or the tetraploid number of chromosomes.

In sections of the sarcoma it was much more difficult to count the number of chromosomes, either in the mononuclears or in the spindle cells, owing to the more compact shape of the cell and the imperfect fixation of the chromosomes. However, it was evident that the spindle cell contained many more chromosomes than the mononuclear and in those cells in which the chromosomes could be drawn and counted the number agreed with that found in the cultures, *i.e.*, 42 in the mononuclear and 84 in the malignant cell.

A comparison of the mononuclear cells and the spindle-cells at the metaphase, anaphase and after division, both in cultures and in sections of the sarcoma, showed there was always twice as much chromosome material in the mitotic figure of the spindle cell as in the corresponding figure of the mononuclear cell. The single band of chromosomes in the metaphase, as well as the two bands in the anaphase, was twice as wide in the malignant cell as in the mononuclear cell. In other words, the large spindle cell, *i.e.*, the malignant cell, differed from the normal cell, not only in the structure of its resting nucleus but in the fact that during division it contained the tetraploid number of chromosomes instead of the diploid number. The mononuclear, on the other hand, contained the normal diploid number of 42 chromosomes.

Owing to the crumpled shape of the nucleus of the malignant cell, the arrangement of the chromosomes sometimes appeared distorted in the late prophase and early metaphase, but never in the late metaphase or in the anaphase. Also, when living cells were followed through the division, no multipolar or asymmetric mitotic figures were seen and division always resulted in two daughter cells of equal size. In fixed preparations and in sections of the sarcoma, where numerous cells in metaphase and in anaphase were studied, it was seen that the dividing cell had one

spindle and that, while the band of chromosomes was wider in the malignant cell than in the mononuclear, it was just as regularly arranged and there was an equal distribution of the chromosomes to the two poles of the spindle in both types of cells (Figs. 3, 6). Only one abnormal division was found. This was in a section of a very necrotic sarcoma. The cell seemed to be a mononuclear, but it had reached the stage where it was impossible to be certain of which type the mother cell had been. It was partly divided into three cells. One of these seemed to have a double group of chromosomes, while the other two each had one group. W. H. Lewis (1927a, p. 151) states that an instance of tripolar mitosis was observed that resulted in three daughter cells.

In the plate accompanying the present observations an effort has been made to illustrate the difference between the malignant cell and the normal cell, both in regard to the resting nucleus and to the number of chromosomes. Figure 1 shows the large nucleus of the malignant cell. It has a heavy membrane and contains many large nuclear masses, no one of which can be definitely stated to be the nucleolus. Figure 2 shows the chromosomes of such a malignant cell in prophase. There are 84 chromosomes; these are neither shorter nor thinner than those of the normal cell shown in Figures 8, 10 and 12. Figure 13, drawn from a different culture, shows the 84 chromosomes of the prophase of a malignant cell grown in rabbit plasma.

Figures 3 and 6 show the anaphase of a malignant cell and of a mononuclear cell, respectively, the two dividing almost side by side in the same culture. Figures 4, 5 and 6 are mononuclears in the same culture and in the same field as the cells shown in Figures 1, 2, and 3. Figure 4 shows the resting nucleus of a mononuclear and Figure 5 the dividing nucleus in late prophase with 42 chromosomes. Figure 7 is from the resting nucleus and Figure 8 from the prophase of the large spindle cell of the spleen. Figure 9 is from the resting nucleus and Figure 10 from the prophase of the clasmatocyte of the subcutaneus tissue; Figures 11 and 12 are from epithelial cells of the thymus. From Figures 1, 2, 3 and 13 it is evident that the spindle cell of the sarcoma has become changed, not only in regard to the cytological structure of the resting nucleus but also as to the number of chromosomes present during karyokinesis. Figures 4, 5 and 6 show that the mononuclear cell is not simply a small tumor cell containing a reduced number of chromo-

somes resulting from an asymmetrical division, but that it has a nucleus like that present in the mononuclears of normal tissue and contains the normal number of chromosomes. From Figures 3 and 6 it can be seen that cell division in the tumor is not asymmetrical. Figures 2, 5, 8, 10, 12 and 13 show that the chromosomes in the malignant cell are not the result of fragmentation, but are double the number contained in the normal somatic cell of this strain of white rats.

Two other albino rat sarcomata were studied, one, a fibrosarcoma, the other, a spindle-cell sarcoma. The chromosomes were not counted but camera-lucida drawings of many cells in the metaphase and in the anaphase showed that the bands of chromosomes in the dividing malignant cells were twice as wide as those in nearby mononuclear cells in the same stage of division and equally as wide as those in the metaphase and in the anaphase of the malignant cell of sarcoma No. 1. In these sarcomata no multipolar or asymmetrical divisions were seen.

DISCUSSION

In animals and plants a certain number of individuals have been found that have double the number of chromosomes characteristic of the species. These are called tetraploids. In these forms the cells are larger than those of the diploid variety. Morgan (1926), in discussing this question, seemed to think that in monoecious plants new types may arise in this way, but that in animals with separate sexes tetraploids could not become established (except in parthenogenetic species) because the tetraploid would be lost by crossing to an ordinary individual. Just how these tetraploid forms arise is a question of speculation. Morgan suggests that they might arise through the fusion of two cells or by the failure of the cytoplasm to divide after the division of the chromosomes. Naturally, one immediately thinks of such a possibility in regard to the origin of the sarcoma cell, but observations made of the behavior of cells in tissue cultures rather lead one to believe differently; for while cells have been observed to fuse, the fusion took place only in the cytoplasm and never between the nuclei. Moreover, as has been shown by Lambert (1912) and by W. H. Lewis (1927b), the fusion resulted in a cell with two or more nuclei and not in a cell with a double-sized nucleus.

Of the thousands of living cells followed during division in cultures of

normal tissues of various kinds, only one normal cell was ever seen in which the cytoplasm failed to divide after the chromosomes had separated. In this instance two separate nuclei were formed and, presumably, had this cell divided by mitosis, two spindles would have occurred, each with its own group of chromosomes. Binucleated cells do occur rather frequently in tissue cultures, and during the division two spindles arise, each with its own group of chromosomes. The division usually results in four cells, but in some instances giant cells containing four nuclei are formed. At rare intervals, extending over many years of observation of living cells in tissue cultures, a cell has been seen to form with a single nucleus containing double the number of chromosomes (Lewis, 1927a). Such nuclei always arose during the mitotic division of a binucleate cell in which the two spindles became so tilted at opposite angles that their corresponding poles approximated. When the chromosomes passed to these poles they became so massed together that one nucleus formed about the two groups. In cultures of normal tissue, where such a division was followed in the living cell, it resulted either in three cells, two of them normal and the third larger with a double-sized nucleus, or in two cells, one binucleate and the other with a double-sized nucleus.

In 1914 Theodor Boveri published his paper entitled "Zur Frage der Enstehung maligner Tumoren." He based his theory on observations of pluripolar mitoses in the eggs of the sea urchin. He found that eggs having double spindles could produce normal larvae, that eggs having tripolar spindles produced some normal and some abnormal larvae, while eggs with tetrapolar spindles produced only abnormal larvae. From this he reasoned that the malignant cell might have such an origin from a pluripolar mitosis. He believed that the cause of the abnormal behavior of the tumor cell lay in its own constitution and not in its environment. According to this author, the malignant cell would have an increased but not necessarily a tetraploid number of chromosomes.

Morgan seems to think that the tetraploid egg would continue to divide into cells with double the number of chromosomes and result in a new type. Certainly the spindle cells of the Walker sarcoma No. 1 appear to have divided in this way. Examination of sections of this tumor made in 1926, 8 months after its appearance as a spontaneous

tumor, shows that, even at this time, both the mononuclear cell and the malignant cell divided with one spindle, and that an equal distribution of chromosomes went to the daughter cells; but that, while the mononuclear had the normal number of chromosomes, the malignant cell had double that number.

Since asymmetrical divisions were not seen in any of the rat sarcomata studied, it is not necessary to go into the discussion between von Hansemann and Stroebe as to the origin of the cancer cell through asymmetrical mitotic division. Neither need we discuss Whitman's views as to somatic mutations as a factor in the production of cancer, since his suppositions are based on von Hansemann's explanation of asymmetrical mitosis as due to delayed or absent longitudinal splitting of the chromosomes The asymmetrical division supposedly results in one cell with a smaller number and one with a larger number of chromosomes. According to this theory, the cell with the larger number of chromosomes becomes the tumor cell, while the one with the smaller number dies. The number of chromosomes in each malignant cell would not necessarily be the same, but would vary according to the conditions in the particular asymmetric mitotic division producing it. In the transplantable Walker rat sarcoma the number of chromosomes in the larger cell and in the smaller cell is definite. The smaller cell is not a small cell with a reduced number of chromosomes resulting from an asymmetrical mitotic division of the malignant cell but is a definite type of cell, the mononuclear. It does not die out but continues to divide and gives rise to mononuclear cells.

Swezy (1928) reported that in a mixed rat strain bred from the Wistar strain of white rat and the ordinary wild grey rat, she found two types of secondary spermatocytes. One contained 21 chromosomes and the other 31. The somatic cells of these rats uniformly contained 42 or 62 chromosomes, but the number of chromosomes for the somatic cells of one individual was constant and no cells were observed with 52 or 84 chromosomes. The results of Swezy suggested the possibility that the malignant cell might have 62 chromosomes but the counts showed there were always more than that number.

Fischer (1927, p. 372) claims to have observed a process akin to conjugation on the part of two sarcoma cells which immediately afterwards divided, but this can hardly have taken place by mitosis,

since Fischer reported that he had never observed mitosis in sarcoma tissue.

In the light of these earlier observations, our findings still fail to be explained. The malignant cell, as found in the Walker rat sarcoma No. 1, seems to be a definite entity; that is, it is no longer being formed, but is a changed cell with not only an increased number of chromosomes but a fixed number. This cell continues to propagate itself by mitotic division. Its origin cannot be explained on the basis of fragmentation of the chromosomes or asymmetric division. It might arise from the division of a cell containing two nuclei, each of which forms its own spindle during division, and in which these spindles become so placed that a pole of one spindle lies so close to the corresponding pole of the other that the two groups of chromosomes arriving at these poles become included in the same nuclear membrane. This leaves the origin of the cell with two nuclei still unexplained. The probability that it arises by fusion rather than by the failure of the cell to divide following the division of the chromosomes is suggested by the observations of W. H. Lewis (1927b), who obtained multinucleated giant epithelioid cells in cultures of this sarcoma in the second replantation in homoplasma. But, whatever the origin of the malignant cell may be, it is interesting to note that in the particular tumor under discussion it carries with it, as one of its attributes, a tetraploid number of chromosomes.

CONCLUSION

The large spindle cell of the Walker sarcoma No. 1 is apparently the malignant cell. It has a peculiar large granular nucleus with a distinct nuclear membrane and during division, which takes place by mitosis, approximately 84 chromosomes appear. The mononuclear cell present in the tumor does not differ from that found in other tissues. It divides frequently by mitosis and the number of chromosomes present at this time is about 42, the same as that found in the somatic cells of the normal albino rat.

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PLATE 1

All figures are from cultures of albino rat tissue, magnified ×2180.

FIG. 1. Resting nucleus of a spindle cell of sarcoma in chicken plasma.

FIG. 2. Chromosomes (84) of prophase of spindle cell of sarcoma in chicken plasma.

FIG. 3. Chromosomes of anaphase of spindle cell of sarcoma in chicken plasma.

FIG. 4. Resting nucleus of a mononuclear cell of sarcoma; same culture as Figures 1, 2 and 3.

FIG. 5. Chromosomes (42) of prophase of mononuclear cell of sarcoma; same cultures as Figures 1, 2 and 3.

FIG. 6. Chromosomes of anaphase of mononuclear cell of sarcoma; same culture as Figures 1, 2 and 3.

FIG. 7. Resting cell of a spindle cell of spleen in chicken plasma.

FIG. 8. Chromosomes (42) of prophase of spindle cell of spleen in chicken plasma.

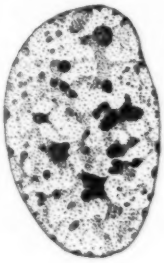
FIG. 9. Resting nucleus of a clasmatocyte of connective tissue in rat plasma.

FIG. 10. Chromosomes (42) of prophase of clasmatocyte of connective tissue in rat plasma.

FIG. 11. Resting nucleus of an epithelial cell of thymus in rat plasma.

FIG. 12. Chromosomes (42) of prophase of epithelial cell of thymus in rat plasma.

FIG. 13. Chromosomes (84) of prophase of spindle cell of sarcoma in rabbit plasma.



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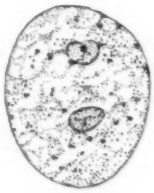

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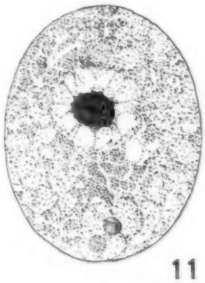

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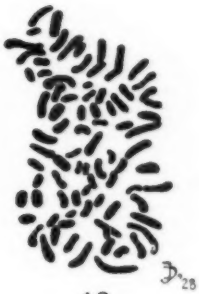

13

VARICELLA COMPLICATED BY ACUTE NEPHRITIS

Report of a Case Associated with Streptococcal Infection of the Tonsils[1,2]

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INTRODUCTION

In 1884 Henoch (1) first observed and reported cases of acute nephritis occurring during the course of varicella. From his observations he concluded that the nephritis was the result of the infectious disease. In the ensuing forty-four years reports upon 52 cases of varicella accompanied or followed by acute nephritis appeared in the literature. That the association of the two diseases is infrequent has been fully recognized. In 200 consecutive cases of varicella studied by Cassel (2) for the purpose of determining the incidence of acute nephritis, the latter occurred but once.

Howland states that, when nephritis complicates varicella, it usually appears late in the course of the disease. It may occur, however, at any time, even during the first week (Beardsley (3), Henoch (1), Morquio (4), and Jansen (5)). Hoenel (6) observed a case in which a mild form of acute nephritis was evident ten days before the appearance of vesicles; and Morquio (4) reported a case associated with cervical adenitis in which the nephritis antedated the eruption by five days. It would seem unlikely in these two cases that the varicella had anything to do with the antecedent acute nephritis.

CASE REPORT

E. K., white male, aged 6, was admitted to the Johns Hopkins Hospital Surgical Service, January 18, 1928, complaining of pain in the abdomen.

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[2] Aided by a grant from the Ella Sachs Plotz Foundation.

[3] Jacques Loeb Fellow in Medicine.

The patient had always been well except for occasional colds and sore throats. Of the infectious diseases he had had only pertussis and that at the age of two or three. A brother, living in the same house was convalescing from varicella at the onset of the patient's present illness.

On January 8, 1928, the patient was found to have a cold and sore throat with swelling of the glands of his neck, from which he recovered after a few days. On January 12, he appeared to be quite listless throughout the day and during the night was seized with a severe abdominal pain, accompanied by distressing nausea. On January 13, the pain, previously in the epigastric region, became localized in the right lower quadrant. Both the pain and nausea persisted but with lessening intensity. On January 17, because of alarming apathy and the fact that his mother thought he was feverish, he was brought to the hospital.

On admission the temperature was 100.2°F., pulse 118, respirations 22. He was a well nourished and well developed boy for his age. There were no abnormalities noted about the head, eyes, ears, nose or mouth except that the tonsils were large; but they were not hyperaemic or oedematous. There was considerable hypertrophy of the pharyngeal lymphoid tissue. No abnormalities were noted about the neck. There was no enlargement of the peripheral lymph nodes. The heart and lungs were normal.

The abdomen was moderately distended; there was cutaneous hyperaesthesia and diffuse abdominal tenderness most marked in the right lower quadrant, where there was definite muscle spasm. Rectal examination elicited marked tenderness. The genitalia, extremities and reflexes were normal. The urine showed no abnormality. There was a leucocytosis of 16,000.

A diagnosis of acute appendicitis with perforation was made. Immediate laparotomy revealed a perforated appendix and localized peritonitis. Cultures from the appendix showed a heavy growth of *B. coli communis;* those from the peritoneal cavity were sterile. Convalescence from the operation was uneventful. A second urine examination on January 19 showed no abnormality. However, on January 27, the temperature rose to 100°F., and on the following day reached 101°F., the tonsils and pharyngeal lymphoid tissue were red and slightly oedematous with wide crypts plugged here and there with fine grayish exudations.

On January 29, there was a profuse eruption with lesions characteristic of varicella. His temperature was 103°F., pulse 120; respirations 30; blood pressure 85/50. He did not appear acutely ill. Over the skin of the trunk, back, chest, arms, legs, palms of the hands and soles of the feet there was a profuse macular, papular and vesicular eruption. A few lesions were seen on the scalp and in the mouth. The largest lesions measured 2 to 3 mm. in diameter and were surrounded by a faint areola. All stages of development characteristic of varicella were present.

The following positive physical findings were noted; enlarged, reddened, oedematous tonsils with wide crypts plugged by exudations, hyperaemia of the pharynx; enlarged, somewhat tender peritonsillar lymph glands; a draining wound, the result of laparotomy.

Urine: Slightly cloudy; s. g. 1.011; acid; sugar 0; albumin +; in the sediment there were a few leukocytes, many red blood cells and a few cellular and leukocytic casts.

Chart I shows the general course of the disease, with the amount of albumin, blood, casts in the urine, charted in relation to the bacteriology of the tonsils and urine.

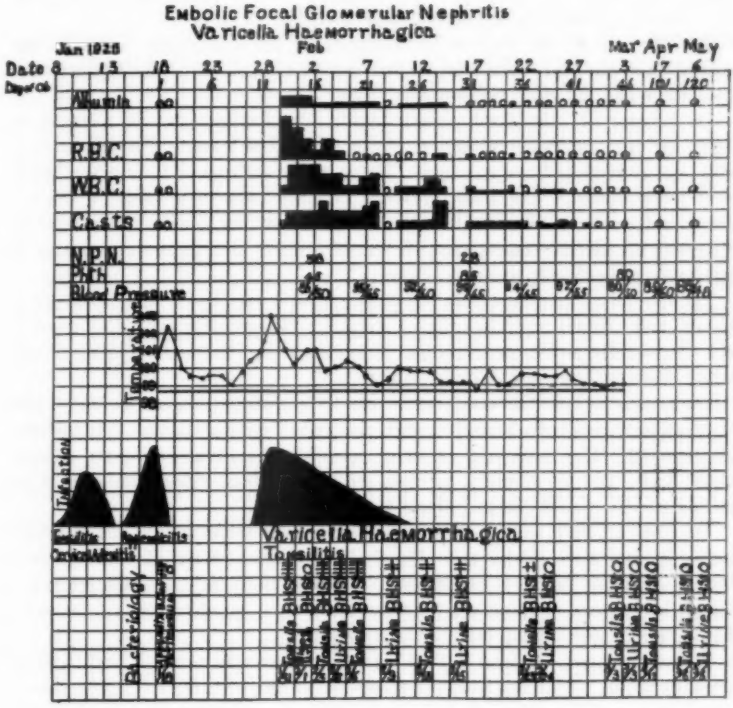

CHART I

The ocular fundi were normal, there was no oedema.

There was a 45 per cent excretion of phenolsulphonphthalein in two hours; the two-hour test of renal function showed a specific gravity variation of 1.010 to 1.020. The blood non-protein nitrogen was 38 mgm. per cent.

On January 31, cultures from the tonsils showed β haemolytic streptococci in great numbers. Cultures from the blood and urine showed no growth. Wassermann test negative. Hg. 85 per cent; R. B. C. 4,300,000; W. B. C. 9,120.

On January 30, many of the vesicles had become haemorrhagic. On February 3, a second urine culture showed enormous numbers of β haemolytic streptococci. On February 6, the eruption was rapidly disappearing, the temperature rising daily only to 100°F. There was slight swelling and redness of the left tonsil, cultures from which showed many β haemolytic streptococci. The ocular fundi were normal; there was no oedema. A third urine culture on February 9 showed many β haemolytic streptococci, the number, however, being less than on February 3. The urinary findings had remained unchanged.

By February 11 the eruption had disappeared, leaving a slight brownish discoloration of the skin. The temperature was 99.4°F., W. B. C. 8,000; there was no oedema and the blood pressure was 95/65.

On February 17, the blood pressure was 95/65; blood non-protein nitrogen 28 mgm. per cent; 'phthalein excretion, 85 per cent in two hours. The tonsils were still slightly reddened and enlarged. There was no oedema and the ocular fundi were quite normal. The urine continued to show traces of albumin, a few red blood cells, and red-blood-cell casts; cultures from the tonsils and urine, on February 13 and 15 respectively, showed β haemolytic streptococci, though in smaller number than in previous cultures.

On February 23 cultures from the tonsils showed very few β haemolytic streptococci and cultures from the urine on February 24 were negative. Coincidently the albuminuria and haematuria had disappeared and the number of casts and white blood cells had markedly diminished.

On March 3, 1928 the temperature was 99°F., blood pressure 80/55. There was no oedema; cultures from the tonsils and urine were negative for β haemolytic streptococci. The 'phthalein excretion was 80 per cent in two hours. Urine: clear; acid; 1.008; no albumin; no casts, red blood cells or white blood cells in the sediment. He was discharged March 6, 1928.

On April 17, 1928, he was seen in the Out-Patient Department. He had been perfectly well since discharged. Physical examination revealed blood pressure 95/60; normal ocular fundi; no oedema. Cultures from the tonsils showed only a few β haemolytic streptococci. Urine: clear; acid; no albumin, no casts, red blood cells or white blood cells in the sediment.

He was last seen on June 6, 1928, and was apparently perfectly well. Blood pressure 88/48. Cultures from the tonsils and urine were negative for β haemolytic streptococci. Urine: clear; acid; no albumin, no casts, no red blood cells or white blood cells in the sediment.

Bacteriology. At frequent intervals, as noted above, cultures from the tonsils and the urine were made, blood-agar poured plates being employed. Haemolytic streptococci of the β type were isolated both from the tonsils and urine. Twenty strains were subjected to fermentation tests, 1 per cent of the following sugars in infusion bouillon being used:—lactose, mannite, salicin, dextrose, raffinose, saccharose, starch and inulin. Seven strains isolated from the tonsils on January 31, February 3, February 6, and February 13, and five strains isolated from the

urine on February 3, 1928, fermented lactose, salicin, dextrose, saccharose and starch, but failed to ferment mannite, raffinose and inulin. From this study one may conclude that the organisms isolated from the tonsils and urine possessed similar properties as regards the fermentation of several sugars.

DISCUSSION

The rôle of streptococci as an aetiological factor in acute glomerular nephritis was suggested first by Lohlein (7). This conception has been elaborated by a number of workers and most recently by Longcope and others (8), whose work, in a large proportion of cases, shows clearly the relationship in acute infections of haemolytic streptococci to nephritis.

The case reported here is one of mild acute nephritis occurring during the course of haemorrhagic varicella and preceded by an acute infection of the tonsils with haemolytic streptococci of the β type. As pointed out above, nephritis rarely occurs during the course of varicella and it is worthy of emphasis that, in this instance, it came on during the relatively infrequent haemorrhagic type of this eruptive disease. Without a careful bacteriological study of the fauces and urine it might have been supposed that the haemorrhagic nephritis was in some way directly associated with the attack of varicella, but the presence of a complicating acute tonsillitis due to β haemolytic streptococci, and the occurrence of the same organism in the urine, during the entire acute phase of the nephritis, leads to the suggestion that the nephritis arose as a complication of the tonsillitis rather than of the varicella. Though it seems probable that the organisms must have reached the kidneys by way of the blood stream, it is of interest to note that a blood stream infection was not demonstrable by blood culture during the early stage of the streptococcus infection. The close parallelism between the course of the nephritis and the course of the infection with the presence of β haemolytic streptococci in the throat and urine lends further support to the view that the nephritis was caused directly by the streptococcus. It is possible also that the secondary infection by streptococci may have been responsible for the haemorrhagic form of the varicella.

Four cases reported by Cassel (2), Morquio (4), Beardsley (3), and Blühdorn (7) of acute nephritis complicating varicella were also

accompanied by infections of the upper respiratory tract. In Blühdorn's case streptococci were recovered from the blood stream.

Certain clinical findings commonly encountered in acute glomerular nephritis were absent in the case reported here. There was no oedema, the blood pressure was never elevated and there were no abnormalities in the ocular fundi. Based upon the urinary findings the case may be classified as one of acute haemorrhagic nephritis, but the progress of the disease and the actual presence of streptococci in the urine indicate that the case was one of focal or possibly embolic nephritis, and not one of diffuse glomerular nephritis such as complicates scarlet fever or occurs during the course of other forms of streptococcus infection.

SUMMARY

1. A case of focal glomerular nephritis occurring early in the course of haemorrhagic varicella is reported.

2. The onset of the nephritis, and the recovery from this complication paralleled the course of a complicating acute tonsillitis due to haemolytic streptococci, of the β type.

3. The organisms isolated from the tonsils and urine had common cultural characteristics.

4. During the acute stage of the nephritis haemolytic streptococci of β type were found in the urine, while after recovery from the nephritis they disappeared both from the throat and the urine.

5. It is suggested that the acute haemorrhagic nephritis in this case was associated not with haemorrhagic varicella but with the streptococcal infection.

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ON PHOTOGRAPHING GROSS PATHOLOGICAL SPECIMENS[1]

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The following notes are offered not to professional photographers, who would certainly sniff at them, but to pathologists for whom the accurate recording of the appearance of hearts, lungs, or other organs from the autopsies is of importance. They are the result of an effort to overcome the difficulties which we have always experienced and to reduce the procedure to such a simple and completely standardized set of manoeuvres that anybody can with perfect regularity make extremely good pictures which may be attached to the autopsy protocol.

Tissues fixed by Kaiserling's method are photographed under water in a tank covered with plate glass, and a small label with the typewritten number of the case, laid on the glass, is included in the photograph. Naturally the proper dissection of the organ and the best possible posing will prove to have been essential when the finished photograph comes to be examined.

This paper cannot deal with the choice of subject nor with the technique of cutting smoothly such organs as the heart or lungs in exactly the right plane, nor with the posing of the organ for the photograph.

But the posing which was formerly an interminable task, requiring stitches and props and clamps stretched by weights to hold the organ in place, is rendered quick and easy by an apparatus which we have devised and had made by G. Wahmann, of Baltimore (Figs. 1, 2 and 3). It is constructed on the principle of the ordinary scale with two pans, one of which goes up when the other goes down. The support of these pans remains vertical because the "beam" beneath is really a hinged parallelogram. Therefore, in the present apparatus which may be understood from the photographs, one pan is represented by a platform, the other by a handle which can be pressed down and

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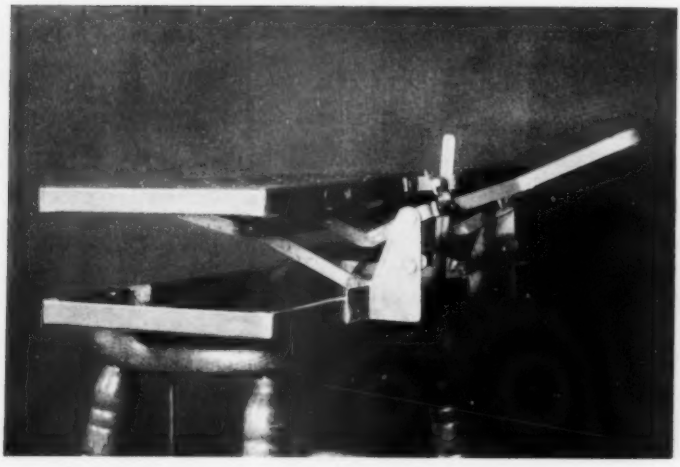

FIG. 1

FIGS. 1, 2 AND 3. DIFFERENT VIEWS OF MOVABLE OBJECT HOLDER OR PLATFORM FOR USE IN COPPER TANK

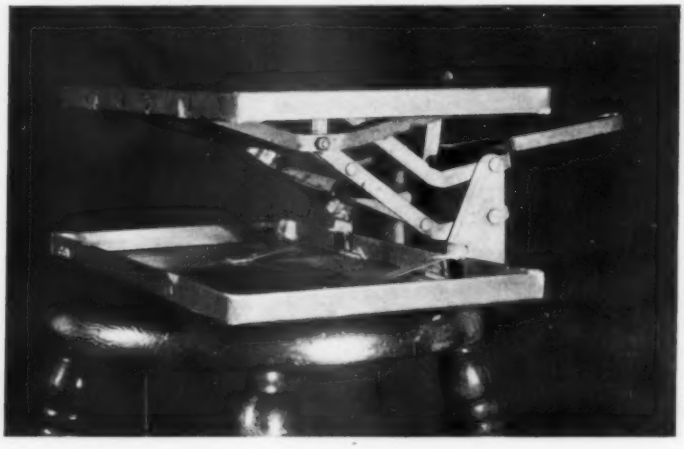

FIG. 2

fixed by a set screw in any position so that the platform is pressed up and fixed in that position. The square tank which we have had for a long time was, I believe, devised by Dr. McCordock and is made of copper, half of it being covered in with plate glass, the other half, which is built up several inches higher, being open, so that when it is filled with water the level of the water is higher than that of the glass. Formerly, we passed the specimen down through the water and tried to fix it in position on a support beneath the glass, but the balance-like apparatus is now lowered through the open half and pushed far under

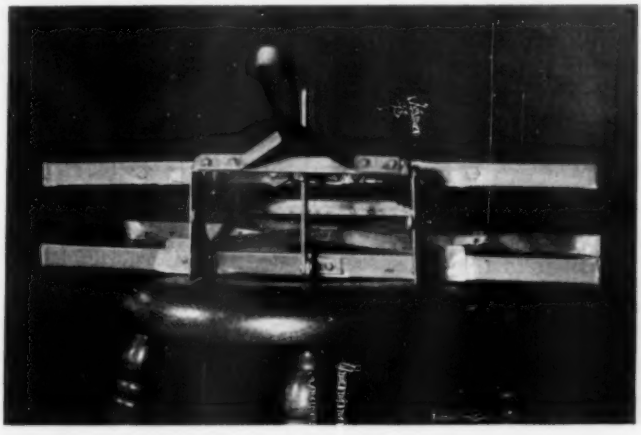

FIG. 3

the glass-covered half where its bottom part hooks under two projections soldered to the back wall of the tank. Then by putting the specimen on the platform and pressing on the handle, it is brought up under water to press against the plate glass and is almost without any further effort spread out and held in position to be photographed from above. All this requires only a moment, and when the number, cut from a card upon which the numbers of all the specimens to be photographed have been typewritten, is laid on the top of the glass, the exposure can be made.

Of course it is plain that the camera is arranged on one of the regular

stands so as to point down and the copper tank is on a very low stand and pushed under it. Then there must be a light on each side and there must be an appropriate background for the specimen. The commercial stands are made so that the camera can be raised or lowered by a long screw—and the focussing is simple. For the background we had at first either white or black glass cut in plates large enough to cover the platform of the apparatus, that is 9 × 17 inches, but it was soon found that neither pure white nor black made the most useful background, for white edges such as those of cartilage became indistinct on a white background and red edges were almost equally so on a black background. Since it is not perfectly easy to foretell the result of photographing any given color through a yellow screen, we photographed in that way the advertising sheet of a paint shop with its little strips of every color numbered and labeled to represent the cans of paint that correspond. Various grays would do and some blues, but it seemed that the best result would be gotten with a background of a rather light fawn color. Accordingly, a sheet of glass was painted with this paint and ultimately the painted side covered with another sheet of glass fixed to it with waterproof cement. This proved an ideal background, giving a pale gray in the photograph which contrasts sufficiently with anything met with in pathological specimens. Since then we have been able to buy from Leo Popper and Sons, 143 Franklin Street, New York, some sheets of glass of exactly this color, but the glass was made for some other purpose in Belgium and their remaining stock is very small. We have tried glass backgrounds of other colors as we chanced to be able to get them, but red, blue and green were unsatisfactory, all printing out black—and sheets of aluminum and other metals were equally unsatisfactory because every tiny scratch showed distinctly in the picture.

Light is obtained from two Mazda bulbs in metal shades on stands which can be moved and upon which the lights can be raised, lowered, or tilted. On the right-hand side of the tank the lamp is one of 1000 watts—on the left of 300 watts. Between the lamp and the tank on each side a sheet of tracing paper is hung on an adjustable stand so that all the light which reaches the object comes through thin tracing paper (Fig. 4). This gives a much softer and more pleasing picture with less disturbing shadows and clearer detail than when uncovered

lights are used. The stronger light is set quite near the tank and about 15 inches above it and kept in this position. The 300 watt lamp on the other side is moved as near to its curtain of tracing paper as is possible without creating a shadow on the other side of the object. It serves to light the part of the specimen nearest it but it must not be near enough to cast disturbing shadows. Air bubbles can be raked

FIG. 4. SHOWS THE TANK WITH OBJECT HOLDER IN PLACE, TOGETHER WITH THE CAMERA LIGHTS AND PAPER SCREENS

out with an automobile windshield scraper fixed in an appropriate handle, or sucked out with a long pipette supplied with a rubber bulb. Little trouble is experienced if the water for filling the tank is kept for a day in an open container after it is drawn from the tap.

We use 5 × 7 plates, and most objects can be accommodated upon them when the lens is about 18 to 21 inches above the surface of the plate glass, the lens in our camera being by Carl Zeiss, Series 1 C,

1:43, F = 18 cm. Wratten and Wainwright panchromatic plates give by far the best results. The loading of plate-holders, development and fixation must be done with a Wratten special series 3 green safelight.

No color screen is put into the camera when these plates are used with the Mazda lamps. After focussing with open diaphragm, the diaphragm is shut down to 1/16, and an exposure of 20 seconds with this arrangement is normal. Of course, if, for the purpose of enlarging, the camera is brought nearer the object and the diaphragm still further narrowed, a longer exposure is necessary.

The exposed plate is developed for three minutes under the Wratten green safelight in the following developer which is good for plates, lantern slides and Azo printing paper.

Stock solution

Dissolve in the order named:	
Water at 52°C.	1,420 cc.
Elon	7.1 grams
Sodium sulphite	106.3 grams
Hydroquinone	28.3 grams
Sodium carbonate	148.8 grams
Potassium bromide	3.9 grams
then add	
Methyl alcohol	184.6 cc.

For use take stock solution 1 part, water 3 parts.

It is important before developing to immerse the plate in running cold water and rub off the surface with the fingers to dislodge air bubbles. After three minutes' development the plate is again washed in water and placed in the acid hypo bath until quite cleared. It is then washed in running water for about half an hour, or more, and before being taken out to dry, both sides are carefully rubbed off with the fingers.

The acid hypo bath, as recommended by the Eastman Company, is as follows:

Water	2 liters
Hypo	480 grams

When thoroughly dissolved add the following solution, each ingredient of which must be completely dissolved before the next is added:

Water at 52°C	160 cc.
Sodium sulphite	30 grams
Acetic acid (28 per cent)	96 cc.
Powdered potassium alum	30 grams

(To make 28 per cent acetic acid dilute three parts glacial acetic acid with eight parts of water.)

Prints are made on glossy Azo paper and developed and fixed in the same solutions as the plates, with the same precautions as to washing, etc., but this may be done in a red or even a yellow light. They are developed until the optimum appearance is reached which takes about $1\frac{1}{2}$ minutes. After washing, they are carefully lowered edge first beneath the surface of the hypo in which they remain for half an hour or less, are then washed about as long and finally spread face down on a clean squeegee board, covered with a large blotter and rolled smooth with a rubber roller. The blotter is removed and they are left to dry. The face of the squeegee board should at intervals be rubbed with a pledget of cotton moistened with an extremely dilute solution of beeswax in xylol, and this rubbed off with a fresh pledget with pure xylol and then with a dry towel until it is quite glistening and smooth. If too much beeswax is left, it marks the prints—if there is too little, they stick fast.

The Azo paper is made in several grades from F 1 to F 5, the first being far more sensitive to light than the last. A normal negative with pleasing contrast of light and shadow gives a good print with F 1, exposed for 2 seconds under a 100 watt Mazda lamp placed about 15 inches above the printing frame, but if the negative is flat, with little contrast, a partial remedy may be found by using F 4 paper and printing with the same light for 4 to 6 seconds. It must be a very poor negative that requires printing with F 5 paper which tends to produce prints in absolute black and white.